The Relationship Between Acid and Neutral Protease Activities and the Incidence of Soft Cooked Texture in the Muscle Tissue of Pacific Hake *Merluccius Productus* Infected with *Kudoa Paniformis* and/or *K. Thyrsitis,* and Held for Varying Times Under Different Pre-Freeze Chilled Storage Conditions.

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ABSTRACT

Tsuyuki, H., S.N. Williscroft, Z. Kabata, and D.J. Whitaker. 1983. The relationship between acid and neutral protease activities and the incidence of soft cooked texture in the muscle tissue of Pacific hake (Merluccius productus) infected with Kudoa paniformis and/or K. thyrsitis, and held for varying times under different pre-freeze chilled storage conditions. Fish. Mar. Serv. Tech. Rep. No. 1130.

One of the more intractable quality problems associated with Pacific hake is the relatively high incidence of development of soft texture during the cooking process. The association of this texture problem with proteolytic activity or with the presence of parasite has not been clearly understood despite long years of study.

In this investigation strong proteolytic activities have been demonstrated in the acid and neutral pH ranges. The proteolytic activity in the acid range has been found to increase greatly in muscle tissues of Pacific hake infected with Kudoa thyrsitis from both the Strait of Georgia and the west coast of Vancouver Island. This enzyme(s) is heat labile and does not appear to degrade those structural proteins responsible for maintaining firm texture after cooking. Pacific hake which are uninfected and those infected with K. thyrsitis do not result in unacceptably soft cooked flesh texture.

Muscle tissues exhibiting strong proteolytic activity in the neutral pH range developed unacceptably soft or mushy texture during the slow cooking process. The strong neutral protease activity was associated with muscle tissues infected only with the white pseudocysts or the young forms of K. paniformis either alone or as a mixed infection with K. thyrsitis. The enzyme(s) was found to be relatively heat stable with an activity optimum at 55°-60°C, a transitional temperature in the cooking process.

The parasite K. paniformis is found only in hake from the west coast, indicating insignificant mixing of the two populations. The development of black pseudocysts in older infections by either Kudoa species is considered a defence mechanism of the muscle tissues. This process deposits melanin granules which eventually lead to the destruction of the parasite and the associated protease activity.

While acid as well as neutral protease activities in the muscle tissues

increase directly with the intensity of the younger form of infection with K. paniformis, increased activity in the acid pH range can be caused by infection with K. thyrsitis alone. Proteolytic activity measured in the acid range therefore is not necessarily associated with the development of soft texture. Failure to distinguish not only the difference between the two parasites but also their age will lead to inconsistencies in relating the development of soft texture to the presence of parasite.

Hake stored with good icing and RSW practices maintain quality for up to 5 days. Subsequent development of soft texture after slow cooking is related primarily to the presence of the parasite K. paniformis.

RÉSUMÉ

Tsuyuki, H., S.N. Williscroft, Z. Kabata, and D.J. Whitaker. 1983. La relation entre l'activité protéolytique en milieu acide et neutre et l'incidence d'une texture molle à l'état cuit, dans le tissu musculaire de la merluche du Pacifique (Merluccius productus) infectée de Kudoa paniformis et/ou K. thyrsitis, et gardée pendant des périodes variables sous différentes conditions d'entreposage à froid avant la congélation. Fish. Mar. Ser. Tech. Rep. No. 1130.

L'un des problèmes les plus difficiles à résoudre quant à la qualité de la merluche du Pacifique est celui de l'incidence relativement élevée de chair à texture molle au cours de la cuisson. Malgré de longues années d'études l'association de ce problème de texture à l'activité protéolytique ou à la présence de parasites n'a pas été comprise clairement.

Dans cette étude on a démontré des activités protéolytiques prononcées au pH acide ou neutre. On a trouvé que l'activité protéolytique dans le milieu acide augmentait fortement dans les tissus musculaires de la merluche du Pacifique infectée par le Kudoa thryrsitis, autant dans celle pêchée dans le détroit de Georgia que celle prise sur la côte ouest de l'île de Vancouver. Cet enzyme(s) est labile à la chaleur et ne semble pas dégrader les protéines structurelles responsables de la préservation de la texture ferme après cuisson. La merluche du Pacifique, qu'elle soit infectée ou non de K. thyrsitis, ne produit pas de chair qui soit molle au point d'être inacceptable après cuisson.

Les tissus musculaires démontrant une forte activité protéolytique en milieu à pH neutre développent une texture molle ou en boullie au cours du processus à cuisson lente. La forte activité protéolytique en milieu neutre était associée uniquement aux tissus musculaires infectés par les pseudocystes blancs ou aux formes jeunes de K. paniformis, soit seule, soit en infection combinée avec K. thyrsitis. L'enzyme(s) s'est montré relativement stable à la chaleur avec une activité optimale à 55°-60°C, correspondant à la température transitoire durant le processus de cuisson.

Le parasite K. paniformis se trouve seulement chez la merluche de la côte ouest, indicant un mélange insignifiant des deux populations. Le développement de pseudocystes noirs dans les infections plus anciennes par chacune des espèces Kudoa est considéré comme un mécanisme de défence du tissu musculaire. Ce processus conduit à la déposition de granules de mélanine qui

causent éventuellement la destruction du parasite et l'activité protéolytique associée.

Tandis que l'activité protéolytique, tant acide que neutre, dans le tissu musculaire augmente directement avec l'intensité le l'infection par les formes jeunes de K. paniformis, une activité accrue dans le milieu à pH acide peut être causée par une infection de K. thyrsitis uniquement. Pour cela l'activité protéolytique mesurée en milieu à pH acide n'est pas forcément associée au developpement de texture molle. Le défaut d'établir une distinction, non seulement entre les deux parasites, mais également entre leur âge, conduira à des inconsistances reliant le développement de texture molle à la présence de parasites.

La merluche entreposée de manière appropriée dans la glace ou dans l'eau de mer refrigérée maintient sa qualité jusqu'à 5 jours. Le développement subséquent de texture molle après cuisson lente est principalement relié à la présence du parasite K. paniformis.

INTRODUCTION

Early interest in Pacific hake (Merluccius productus) centered around the potentiality of developing an industry based on production of fish meal and pet food. A hake meal plant came into operation in 1966 but closed shortly thereafter in 1967 (Dassow and Beardsley, 1973). In a 1977 survey by bottom trawl and hydroacoustic mid-water trawl in the INPFC statistical areas, the resource has been estimated to be in excess of one million tonnes (Dark et al. 1980). The stocks in the Strait of Georgia have been reported to be about 100,000 tonnes (Thompson and McFarlane, 1982). The landings over the past decade in the INPFC statistical areas and off the coast of British Columbia are shown in Table I. The flesh of this species, however, is generally regarded to be of low quality with poor keeping characteristics. Nevertheless the magnitude of the resource stimulated interest since the middle 1960's in increasing utilization, particularly as human food. Some assessment studies were carried out on the quality of flesh under chilled storage conditions (Dassow et al. 1970) and on the stability and desirability characteristics of fillets and minced flesh blocks (Crawford et al. 1972a,b, 1979). Compositional analyses of hake from Puget Sound and the west coast of the United States were carried out (Patashnik et al. 1970). The development of oxidative rancidity in the subcutaneous fatty layer is one of the quality problems encountered during frozen storage. Quality loss of this nature can be minimized by the application of good glazing practices and low frozen storage temperature of -29°C (Dassow et al. 1970) and by the use of antioxidants (Crawford et al. 1972a). However, the more intractable problem impeding wider utilization lies in the significantly high incidence of development of mushy texture in slow cooked fillets (Dassow et al. 1970; Patashnik et al. 1982). Various reports have indicated that the proteolytic activity responsible for the autolysis can be destroyed by rapid cooking (Dassow et al. 1970) such as by microwave (Patashnik et al. 1982) or by deep frying in fat (Crawford et al. 1972a,b, 1979; Patashnik et al. 1982). Common oxidizing agents such as hydrogen peroxide and potassium bromate have been reported to inhibit proteolysis (Spinelli and Steinberg, 1978) but the method has not been used commercially.

The relationship between pre-freeze chilled storage time and quality has not been established with certainty. There appears, however, to be general

agreement that thorough icing or storage of hake in refrigerated seawater immediately after capture is essential, as well as rapid processing to allow freezing within several hours (Dassow and Beardsley, 1973, Dassow et al. 1970; Miyauchi, 1977; Swanson and Ridenhour, 1978).

The development of mushy texture in oven baked fillets from hake caught in the offshore waters of Washington and Oregon has been reported to be associated in some way with the presence of myxosporean parasite in the flesh (Dassow et al. 1970). However even very recently it has been reported that the severity of parasitization in fish as determined by visual presence of parasitic cysts may not correlate with the degree of softening of cooked flesh (Patashnik et al. 1982). The same report also shows that the most severely parasitized fish fillets could have both high and low proteolytic activity as measured with hemoglobin substrate under acidic conditions. Conversely, high proteolytic activity was shown in muscle tissues from fillets showing only trace infection. Clearly, conclusive evidence has not yet been established to relate the development of unacceptably soft texture after cooking with either the degree of parasite infestation or with the extent of proteolytic activity under the conditions measured.

This study was undertaken to examine both the characteristics of the proteases involved in proteolytic activity and the contribution of parasites present in the flesh toward the development of mushy texture in cooked flesh of Pacific hake. The identification and prevalence of parasites in hake from the Strait of Georgia and the west coast of Vancouver Island was carried out at the Pacific Biological Station. The parasitological investigation and the protein and texture studies were conducted on the same fish to provide direct relationship between mushy texture development and the presence of a specific parasite. From this study it has already been revealed that the musculature of Pacific hake is infected with two species of Myxosporea, Kudoa thyrsitis and a previously unknown species which the authors named Kudoa paniformis (Kabata and Whitaker, 1981). The new species was specific only to specimens obtained from the west coast of Vancouver Island.

MATERIALS AND METHODS

Fish used in the study were from two areas of southern British Columbia, the west coast of Vancouver Island and the Strait of Georgia (Fig. 1). The west coast or "offshore" sample was obtained in midwater trawl by M/T Callistratus in 60 fathoms in September, 1980, roughly fifty kilometers south of Cape Beale (48°30'0"N. x 125°15'0"W.). Within one hour of capture 35 fish were filleted and the fillets plate frozen immediately and stored at -28°C to serve as "zero time" samples. A total of 160 fish was immediately chilled on ice; an additional 160 fish were placed in refrigerated seawater (R.S.W.) at a temperature of -1.5° to 0°C. At intervals of from 4 to 120 hours post mortem, 25 fish were removed from each of the two chilled storage systems and filleted. The fillets were immediately plate frozen and stored at -28°C. One of the fillets from each fish was examined by the Pacific Biological Station in Nanaimo for the presence of protozoan parasites. The second fillet was subsequently assayed for autolytic protease activity and the cooked texture was determined. The inter-relationships which existed between the degree of infection by Kudoa, the autolytic activity, and the cooked texture of the fillets were then examined.

Two separate samples of hake were obtained from the Strait of Georgia. The first, in March 1980, was obtained by midwater trawl in 198 fathoms by M/V Ocean King in lower Georgia Strait (49°13'1"N. x 123°34'0"W.). A total of fifty fish was immediately iced and at various post mortem times of up to 3 days, lots of ten fish were removed, plate frozen and stored at -28°C. No parasitological or protease evaluations were conducted on these samples but the textures of the cooked flesh were determined.

A second sample of 100 hake from the Strait of Georgia was obtained in January, 1981, by midwater trawl in 60 fathoms by M/V Sharlene K. approximately 27 km south of Savary Island (49°47'12"N. x 124°48'0"W.). All fish were iced immediately and after 2 days approximately half of the total was filleted, the fillet from one half examined for parasites by the Pacific Biological Station and the other half frozen and held at -28°C for the determination of proteolytic activity. The remaining iced fish were handled and prepared in an identical manner after 3 days icing. Additionally, the cooked textures of all fish were determined.

Parasite Evaluation

For the purpose of ascertaining the prevalence and intensity of Kudoa infections, each fillet was divided horizontally along the lateral line and vertically into three approximately equal parts, so that six areas were identified: area I anterior and above the lateral line, area II below it, area III in the middle and above the lateral line, area IV below it, area V posterior and above the lateral line, area VI below it. Fillets were examined under dissection microscope, directly after defrosting. Each area was macerated, several fibres at a time, and all abnormal fibres were checked under compound microscope, at x900 magnification, with the aid of phase contrast illumination. Numbers of infected fibres in each area were recorded separately. Presence of 20 or fewer such fibres was arbitrarily classified as light infection, between 21 and 100 medium infection and over 100 heavy infection. Spores were studied with the aid of light microscope and with scanning electron microscope. Methods used in that study are described elsewhere (Kabata and Whitaker, 1981).

For the purpose of ascertaining an overall average infection level for each fillet, a mean infection density (MID) was calculated from the data reflecting the intensity of parasite infection in the six sub-sampled areas. An example of its calculation follows:

Each of the six areas (see PARASITE EVALUATION) of each fillet was designated to be infected to a light, medium or heavy degree, if at all.

L = light infection = 20 or fewer infected fibres
M = medium " = between 20 and 100 infected fibres
H = heavy " = more than 100 infected fibres

A typical fillet might be described:

Area	I	II	III	IV	V	VI
Infection	H	M	H	M	M	L

For the purpose of calculation, L was arbitrarily assigned a value of 20, M a value of 60, and H a value of 100. The "MID" for the above fillet would be: 400/6 = 66.7.

Protease Activity

The preparation of homogenates for the determination of total proteolytic activity of muscle against endogenous substrates followed the method of Wojtowiez and Odense (1972). Each fillet was not systematically sub-sampled in the manner described earlier for the parasite evaluation. Instead, four or five sub-sections totalling 5-10 gm were removed from representative areas of each fillet and pooled. The overall proteolytic activity was subsequently determined in duplicate for each fillet.

The determination of autolysis was made according to Lowry et al. (1951) as summarized by McDonald and Chen (1965). The protease activity values represent the extent of proteolysis at a standardized white muscle concentration of 3.5 mg/ml.

Preliminary work on representative numbers of hake established the conditions to be used in the systemic assay of proteolytic activities in the raw fillets. These parameters included incubation time, temperature(s) and pH(s) of reaction and will be discussed in a later section.

Two buffers were used throughout. Reaction medium in the acidic range was maintained by a citric acid-sodium citrate buffer (Biochemists Handbook, Ed. Cyril Long, 1961) and in the neutral and alkaline range with Kolthoff's borax-phosphate buffer (C.R.C. Handbook of Biochemistry. Selected Data for Molecular Biology, ed. Sober, 1968).

Cooking Method

Frozen fillets were thawed by immersion in double polyethylene bags for 1.25 hours in 20°C water. The thawed white muscle was then cut into pieces 5 x 5 x 1.0 cm thick, wrapped in a single layer of aluminum foil and either steamed for 10 min (fast cook) or baked in a pre-heated oven @ 177°C for 25 min (slow cook). The hake obtained in the Strait of Georgia during March, 1980, were cooked by steaming while all other hake were cooked by baking. The cooked muscle was cooled to room temperature prior to evaluation of texture.

Texture

The determination of firmness of the cooked flesh was made with the Ottawa Texture Measuring System (O.T.M.S. Model D-1203). The operational parameters have been detailed previously (Bilinski et al. 1977). The 10% and 20% range selections were used.

RESULTS AND DISCUSSION

I. Parasite Infection

Examination of fillets reserved for parasitological study revealed that the West Coast or offshore hake were infected with two species of the genus Kudoa. One of these species was identified as Kudoa thyrsitis (Gilchrist, 1924). Although the spores found in these fish differed in some details from those originally described as K. thyrsitis, the overall resemblance was close enough to suggest that they belong to that species. K. thyrsitis has been previously recorded from Merluccius capensis, the South African hake (Priebe, 1967). The other species has not been previously known and it is considered a new species. Its description, as well as that of K. thyrsitis, has been published elsewhere (Kabata and Whitaker, 1981). The name given to the new species is Kudoa paniformis.

The Georgia Strait hake harboured only one species, K. thyrsitis. Although an occasional straying of the second species, K. paniformis, might still be discovered, it is now certain that it does not reside in the Strait of Georgia. All information on which this account has been based, comes from a single offshore sample. It is possible, therefore, that more extensive sampling will result in a modification of conclusions presented in it.

The signs of infection produced by Kudoa thyrsitis are very similar to those produced by Kudoa paniformis, so that the identity of the pathogen can be determined only by examination of the spores under compound microscope. The parasite penetrates the core of individual muscle fibres and spreads along them without damaging sarcolemma, until each fibre is filled with spores. These fibres, known as pseudocysts, isolate the parasite, so that the connective tissue of the host is not provoked into reaction. They are

slightly distended and are externally recognizable by their pale yellowish colour. It appears that the only marked defensive reaction of the fish is the deposition of melanin granules around the area invaded by the parasite. The timing of the onset of this reaction and its rate cannot be determined on the basis of the present information. It is clear, however, that the reaction is provoked by the presence of the parasite and that it continues for some time, causing a gradual accumulation of melanin and a corresponding change in the colour of the fibre. Turning initially light brown, it becomes dark brown and eventually black. This melanin deposition has an obviously detrimental effect on the spores, which are progressively compressed, crumpled and, in the end, destroyed.

As mentioned earlier, the offshore population of hake was infected with two species of Kudoa, which occurred separately in different fish or formed mixed infections. Of the 322 fish examined, only 34 (ie. 10.5%) were free of Kudoa. The number infected with Kudoa thyrsitis alone was 104 (32.3%) and with Kudoa paniformis 125 (38.8%). Mixed infections were found in 59 fish (18.3%).

Although nearly 90% of hake were infected, the intensity of infection was not uniform, many fish carrying only a few pseudocysts. The infection was distributed through the thickness of the flesh, infected fibres being located with apparently equal frequency close to the inner boundary of the body wall, deep within the muscle and in subcutaneous layers. The distribution of the parasite in different areas of the body was less uniform, as can be seen in Tables II and III. The infection was generally heaviest in the anterior part of the fillet and tended to diminish in a posterior direction. Pseudocysts of K. thyrsitis were predominantly old, judging from their dark colouration, only relatively few pale pseudocysts being present. This species does not appear to produce heavy infections. Only 3% of areas I carried heavy old infections; the proportion of medium infection was also low, the highest number being 10% for area I. Light infections were much more common (74% in area I), but here also old pseudocysts predominated.

This preponderance of black infected fibres suggests that Kudoa thyrsitis infects hake at a low rate, tending to accumulate black fibres with age but seldom producing very many pseudocysts at the same time. Perhaps this might be taken as an indication of an old and well-established relationship between Merluccius productus and Kudoa thyrsitis.

The situation with Kudoa paniformis is quite different. Figures in Table III show that it produces many more heavy infections and that most of those infections are of recent origin. In the light category, old infections are more common than the recent ones; in the medium category the position is intermediate. These figures create the impression that, although both species of Kudoa might begin to infect hake at about the same time, subsequent course of infection is quite different. Whereas the infection rate with K. thyrsitis diminishes with the age of the fish, that with K. paniformis continues and perhaps even increases. Hence the latter species is capable of producing massive numbers of young, pale pseudocysts. When compared with the infection pattern of K. thyrsitis, that of K. paniformis seems to suggest that it is a more recent parasite of hake, with which it still remains in a rather unstable equilibrium. It is, therefore, more likely to be severely damaging to its host than is K. thyrsitis.

The picture presented by mixed infections is, not unexpectedly, intermediate between that resulting from infections with single species.

Both the juvenile and adult populations of hake in the Strait of Georgia were infected with K. thyrsitis only. As might be expected, the pattern of infection in the adult fish resembles that of the offshore infections with this parasite (Table IV and V). There were relatively more heavy infections, but in all three categories (heavy, medium and light) the old, black pseudocysts predominated over the new ones, the ratio of old to new being roughly 2:1.

II. Protease Activity - temperature and pH effects

Fish muscle proteinases active in the acid pH range have been demonstrated in a number of species of fish (cited in Makinodan and Ikeda, 1969c). Recently a carp muscle cathepsin D was described which hydrolysed myofibrils prepared from the same species at pH 3-4 but not above pH 6.0 although some hydrolysis occurred at higher pH with casein as substrate (Makinodan et al. 1982). The participation of this enzyme in autolysis was considered doubtful as the post-mortem pH of carp muscle ranged between 6.3-6.6 (Makinodan et al. 1980).

Fish muscle proteinases active in the slightly alkaline or neutral pH

range with casein as substrate have been described in a number of investigations (Makinodan and Ikeda, 1969a,b, 1971, 1976; Makinodan et al. 1979; Iwata et al. 1974a,b). These proteases in both the crude and partially purified form possessed optimal activities in the slightly alkaline pH range and at high temperatures of around 60°C with casein substrate and were demonstrated in a number of fish species (Makinodan and Ikeda, 1969a,b; Iwata et al. 1974a,b,c). The alkaline protease from the muscle of a number of fish shows little or no activity against casein at lower incubation temperatures of around 35-37°C (Makinodan and Ikeda, 1969b; Iwata et al. 1974a,b).

A partially purified protease with optimum activities at a pH of 8 and at elevated temperatures was demonstrated in mullet muscle using urea denatured hemoglobin as substrate (Deng, 1981). While the pH optimum of this enzyme was not tested against muscle proteins of mullet, it was noted that significant residual activity existed at pH 6.0, the same pH of mullet muscle. The still significant level of activity at this pH was implicated in the reduction of shear force value of mullet flesh at or near the temperature optimum of the enzyme.

A partially purified neutral proteinase from carp muscle has been described (Makinodan et al. 1979). Although its activity toward carp muscle myofibrils has not yet been demonstrated, it has been stated that endopeptidase activity towards both intact and partially hydrolysed hemoglobin suggests that the neutral proteinase may play a significant role in autolysis of fish meat. The role of the other endopeptidases in carp muscle, cathepsin D and alkaline proteinase, is thought to be difficult to relate to autolysis of fish muscle (Makinodan et al. 1979). Cathepsin D is inactive in the neutral pH range and alkaline proteinase is inactive at temperatures below 55°C. However, the role of alkaline proteinase during the increase in temperature in the slow cooking process is another matter.

Both the pH and temperature for optimal activity of proteases are influenced considerably by the substrate used for measuring the activity of the enzyme. Muscle protease activity measured by splitting of denatured hemoglobin from sources other than fish is invariably greater than that measured by using muscle proteins from the same species as substrate and may not reflect the true activity of the tissue (Wojtowicz and Odense, 1972). Furthermore, fish hemoglobins are not expected to be present in significant levels in the muscle tissues of fish with white flesh. As the development of

mushy texture reflects the hydrolysis of structural proteins of the musculature, it is more meaningful to measure the protease activity against myofibrillar proteins from the same species.

From the present study it is evident that the proteolytic activity of Pacific hake muscle at pH 3.8 is optimal at temperatures between 35-40°C (Fig. 2). This was found in representative numbers of both Georgia Strait hake infected with only K. thyrsitis and west coast hake infected with both K. thyrsitis and the new parasite K. paniformis. The activity at this pH decreased at higher temperatures. Differences in the magnitude of proteolysis existed between samples but the shape of the temperature curves was quite similar.

As the mean pH of unbuffered hake muscle samples was found to be 6.88 with a range of 6.74 to 7.02 protease activity at pH near neutrality was considered relevant to the understanding of texture problems of the musculature at temperatures preceding the inactivation point of the enzyme. At pH 6.7 none of the Georgia Strait hake, whether infected with K. thyrsitis or not, exhibited significant proteolytic activity at any temperature. The protease from west coast hake, on the other hand, displayed a temperature optimum between 55-60°C (Fig. 3) at pH 6.7. Although variations in activity were observed between fish, the shape of the temperature curves was about the same. Where proteolysis is measured by autolysis the activity optima are generally broader than those reported in the literature using single protein substrates.

At 35°C the muscle homogenates of non-infected hake from both the Strait of Georgia and the west coast display optimal proteolytic activity at around pH 4 (Fig. 4).

At 60°C the proteolysis curve for the above samples differed not only by geographic area but also by a shift in the acid protease pH optima from 4 to 4.5 (Fig. 5). This observed shift in optima may reflect factors such as the possible existence in muscle tissues of several acid proteases with different temperature optima or the differential heat stability of non-enzyme and enzyme proteins influencing the composite activity pattern. In the neutral pH range protease activity of uninfected Georgia Strait hake is minimal while that of uninfected west coast hake indicate a second peak of activity (Fig. 5). It is not known at this time whether some protease activity in the neutral pH range is an inherent geographic difference or whether it is a

result of infection with K. paniformis at a level undetectable even by thorough examination.

Because at least some offshore hake possess significant proteolytic activity both in the acid and neutral pH ranges and because literature reports that neutral or slightly alkaline proteases may be implicated in the autolytic mechanism, all samples were routinely measured for proteolysis at pH 3.8 and 35°C for acid protease and at pH 6.7 and 60°C for neutral protease.

III. Strait of Georgia Hake

A. Protease Activity

Strait of Georgia hake heavily infected with K. thyrsitis exhibit maximum proteolysis only in the acid pH range whether at 35°C or 60°C (Fig. 6). At the higher temperature, however, maximum proteolysis occurs at a slightly higher pH as was also observed in Fig. 5. When proteolysis is measured at pH 3.8 and 6.7, average activity at the lower pH is considerably higher (Table VI). When the samples from Georgia Strait infected with parasite were divided into those in which the K. thyrsitis existed mainly in the young white pseudocyst form ($W^+B^\pm$) and in the older black pseudocyst form (W^-B^+), significant differences in proteolytic activity can be seen. Muscle tissues infected with young parasite show an average protease activity almost 7 fold higher at the acid pH compared to that at neutral pH. For tissue in which the infection is old, the difference is 3.5 fold.

B. Cooked Flesh Texture

Muscle tissue in which the infection is new show a higher average acid protease value and a lower OTMS shear press force (Table VI) compared to tissue with older infection. However, none of the Strait of Georgia samples were considered too soft or mushy in texture after slow cooking. While acid protease activity may be contributing to some proteolysis at flesh pH of 6.88

the extent is not sufficient to result in texture that is unacceptably soft. The texture of fast cooked samples from an additional 50 hake sampled in March, 1980 was also similar.

C. Relationship of Infection, Protease Activity and Texture

While the young form ($W^{+}B^{\pm}$) of infection by K. thyrsitis was correlated with the acid protease activity [corr. coeff. 0.62 ($p<.01$)] and neutral protease activity [corr. coeff. 0.60 ($p<.01$)], there was no correlation of either protease activity or the presence of the parasite in either form with the firmness of cooked flesh of hake from Georgia Strait. The protease associated with hake from Georgia Strait has very little activity in the neutral pH range (Fig. 6). From the texture standpoint the hake from Georgia Strait is somewhat similar to those described for the same species from Puget Sound. Infection of the Georgia Strait hake with K. thyrsitis does not result in the development of mushy texture during slow cooking.

IV. West Coast Hake

A. Protease Activity

At 35°C west coast hake infected with both Kudoa species or with K. paniformis alone possess protease activity in the acid pH range with an optimum at about 3.8 and very little activity in the neutral pH range (Fig. 7). At 60°C, however, the protease activity is evident over a broad pH spectrum including both the acid and neutral range. The activity of both proteases from infected west coast hake is considerably higher than that observed from uninfected hake from the same area (compare Fig. 5 and 7). The most striking difference in the protease activity profile between infected hake from the two geographic sampling areas is that at 60°C, a transitional temperature in the cooking process, Georgia Strait hake infected with K. thyrsitis has very little activity in the neutral pH range (Fig. 6) while the west coast hake infected with K. paniformis exhibit strong activity in

the same pH range (Fig. 7, Table VI).

The protease activity profile of muscle tissues from west coast hake both uninfected and infected with K. thyrsitis alone is very similar to that of the Strait of Georgia infected with the same species of parasite. Muscle tissues with young and old infection with K. thyrsitis show some difference in acid protease activity but for these samplings at least, not as large as that observed for Georgia Strait hake. It is important to emphasize here that west coast hake in which infection with K. paniformis is old display enzyme activity values for both the acid and neutral proteases somewhat similar to those of uninfected fish and those infected only with K. thyrsitis from both geographic areas.

Muscle tissues with mixed infection show acid and neutral protease values intermediate to those infected with K. paniformis alone or with K. thyrsitis alone.

B. Cooked Flesh Texture

Uninfected west coast hake as well as those infected with K. thyrsitis in either the young or old form are firm in texture after slow cooking (Table VI). Georgia Strait hake infected with K. thyrsitis, while not considered too soft or mushy, gave consistently lower OTMS readings. Inherent geographic differences in the two isolated populations (Kabata and Whitaker, 1981) and the larger size of west coast hake in this study could account for the difference. West coast hake infected with K. paniformis in the young ($W^{+}B^{\pm}$) form gave low OTMS readings after slow cooking and all samples were considered excessively soft and mushy. Those samples infected with K. paniformis in the old ($W^{-}B^{+}$) form did not develop a mushy texture. As stated earlier in the discussion under Parasite Infection, the deposition of melanin granules appears to be a defensive mechanism of the muscle tissues for the process culminates in the destruction of the spores. The low neutral protease activity together with the firmness of the cooked fillets from muscle tissues of west coast hake which has a prevalence of old black pseudocysts also appears to reflect the destruction of the ability of the parasite to produce proteolytic enzymes.

Samples infected with both Kudoa species resulted in varying degrees of softness and mushiness and all were rejected on this basis.

C. Relationship of Infection, Protease Activity and Texture

West coast hake infected with K. paniformis in the $W^{+}B^{\pm}$ form show a strong positive correlation with both neutral protease activity [corr. coeff. 0.79 ($p<.01$)] and acid protease activity [corr. coeff. 0.28 ($p<.01$)] and a strong negative correlation with firmness [corr. coeff. 0.66 ($p<.01$)].

In this investigation the development of soft cooked texture was associated only with west coast hake infected with K. paniformis alone or together with K. thyrsitis. Both Georgia Strait hake, which are infected only with K. thyrsitis, and west coast hake infected with only the same species do not result in the development of mushy texture in cooked flesh.

The relationship of protease activity and autolysis of the musculature is complex. The literature reports that the muscle tissues of many species of fish have protease activity in the acid and slightly alkaline pH ranges. However, detailed examination for the presence of parasites has not been an integral part of those investigations. From the present study it is evident that infection of the musculature of Pacific hake with K. thyrsitis results in a large increase in protease activity in the acid pH range. However, infection with K. paniformis results in a large increase in protease activity in both the acid and neutral range. Whether the protease activities associated with the parasite at these pH ranges are the same as those reported in uninfected fish is not known.

This investigation has revealed that all Pacific hake showing strong protease activity in the presence of parasite at 60°C result in development of mushy texture in cooked flesh. Acid protease activity in muscle tissues contributed by K. thyrsitis does not cause mushy texture at flesh pH although some degree of softening may be occurring. The contribution to mushy texture by acid protease activity in those specimens also showing strong neutral protease activity, as in hake infected with K. paniformis alone, can not yet be ruled out. However, protease from K. paniformis show activity at 60°C over a broad pH spectrum with an optimum near neutrality. It is reported in

the literature that fish muscle acid proteases with pH optima of about 3.8-4.0 are not consistent with the post-mortem pH of flesh which is near neutrality. Makinodan et al. (1982) reported that cathepsin D from carp muscle hydrolyses carp myofibrils at pH 3-4 but not at 6.0. Neutral or slightly alkaline protease activity is more favorably viewed as being involved in the autolytic proteolysis of fish muculature. Acid protease activity in parasite infected Pacific hake muscle is heat sensitive, becoming relatively inactive at the elevated temperatures expected during the cooking process. Hydrolysis which can be demonstrated by protease activity in the infected hake muscle tissue in the acid pH range does not appear to be splitting to any great extent at muscle pH, those structural proteins which maintain firm texture.

V. The effect of post-mortem chilled storage time on the acid and neutral protease activities and its relationship to the development of soft texture.

The effect of post-mortem chilled storage time on ice and in RSW, for up to 120 hours, on the development of soft texture in Pacific hake during slow cooking is shown in Fig. 8 and 11 respectively. Samples were segregated by geographic area and by species of Kudoa with which the fish were infected. The west coast samples were frozen immediately on-board and after various lengths of chilled storage time for subsequent analyses for cooked flesh texture and for proteolytic activities. Immediate freezing was not possible with samples from the Strait of Georgia as the trawlers were not equipped with on-board freezing facilities.

During storage on ice the shear force values of all west coast hake after cooking dropped sharply during the initial chilled storage periods of 4-8 hours and subsequently remained either stable or declined very slowly within the limits of the experiment (Fig. 8).

When the muscle tissues were segregated by the species of parasite present, all west coast hake infected with K. paniformis showed the lowest shear values and the textures after cooking were considered clearly unacceptable beyond 4 hours of chilled storage. These samples, regardless of chilled storage time, up to and including zero time, consistently produced

shear values only approximately half those of fish uninfected or infected with K. thyrsitis alone. None of the latter was judged too soft for up to 120 hours of ice storage.

Chilled storage for up to 120 hours results in cooked flesh of acceptable firmness provided the hake is not infected with K. paniformis alone or in a mixture with K. thyrsitis. Although infection with the former was not separated by young and old forms, it is presumed that those fish with the older form would have a firm texture as indicated in Table VI.

Fig. 9 and 10 show that both the acid and neutral proteases are high in fish infected with K. paniformis and the level of activity decreases slightly with increased chilled storage time. Conversely, west coast hake infected only with K. thyrsitis display lower activities for both proteases, decreasing little or none up to 120 hours. In addition proteolysis appears to be at a virtual standstill throughout the chilled storage period of 120 hours as shown by low blank values (Fig. 14). The presence or absence of K. paniformis, rather than ice storage time to 5 days, appears to be the main determining factor in the acceptability of the cooked flesh from the standpoint of texture.

The results obtained for storage in RSW are essentially the same as for ice storage and only the results are shown (Fig. 11, 12, 13).

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TABLE I. Pacific hake landings (tonnes) 1971-1981.

	INPFC* statistical area	British Columbia Waters**		
		Foreign Vessel	Cooperative Fishery	Domestic
1971	156,592	5,659		
1972	117,506	39,637		
1973	157,964	5,983		
1974	166,900	17,146		
1975	206,466	15,752		
1976	187,381	5,972		
1977	103,200	5,191		
1978	98,500	4,650	1,814	
1979	136,500	7,900	4,233	818
1980	83,500	5,901	13,210	606
1981		3,105	17,239	5,692 (2,410 inshore 3,282 offshore)

* 1971-76 data from Forrester et al. (in press).
1977-80 data from Forrester et al. (personal communication).

** From B. Ackerman (personal communication).

TABLE II. Prevalence of Kudoa infections in hake off the west coast of Vancouver Island, distributed by body area (in absolute numbers).

		I		II		III		IV		V		VI	
		W	B	W	B	W	B	W	B	W	B	W	B
Kudoa thyrsitis	Heavy	-	3	-	-	-	-	-	-	-	-	-	-
(n = 100)	Medium	-	10	-	4	-	7	-	3	-	3	-	1
	Light	11	74	6	57	2	69	3	62	1	58	2	44
	Negative	10		31		23		33		39		53	
Kudoa paniformis	Heavy	59	18	42	13	41	15	35	9	30	8	29	5
(n = 124)	Medium	14	35	13	21	17	20	14	17	15	10	10	9
	Light	25	30	29	37	34	45	33	49	28	51	24	47
	Negative	8		14		12		19		25		36	
Mixed infections	Heavy	16	5	12	2	8	2	5	-	5	-	4	-
(n = 59)	Medium	15	14	8	10	9	10	9	9	7	2	1	3
	Light	18	34	25	36	20	37	23	38	18	38	24	34
	Negative	-		5		3		4		6		15	

TABLE III. Prevalence of Kudoa infections in hake off the west coast of Vancouver Island, distributed by body areas (in % of population examined).[1]

		I		II		III		IV		V		VI	
		W[2]	B[3]	W	B	W	B	W	B	W	B	W	B
Kudoa thyrsitis	Heavy	-	3.0	-	-	-	-	-	-	-	-	-	-
(n = 100)	Medium	-	10.0	-	4.2	-	7.0	-	3.0	-	3.0	-	1.0
	Light	11.0	74.0	6.31	60.0	2.0	69.0	3.0	62.0	1.0	58.0	2.0	44.0
	Negative	11.0		32.0		23.0		33.0		39.0		53.0	
Kudoa paniformis	Heavy	47.6	14.5	35.0	10.8	33.1	12.1	28.2	7.2	24.2	6.4	23.4	4.0
(n = 124)	Medium	11.3	28.2	10.8	17.5	13.7	16.1	11.3	13.7	12.1	8.1	8.1	7.9
	Light	20.2	24.2	24.2	30.8	27.4	36.3	26.6	39.5	22.6	41.1	19.3	37.9
	Negative	6.4		11.7		9.7		15.3		20.2		29.0	
Mixed infections	Heavy	27.1	8.5	21.0	3.5	13.5	3.4	8.5	-	8.5	-	6.8	-
(n = 59)	Medium	25.4	23.7	14.0	17.5	15.2	16.9	15.2	15.2	11.9	3.4	1.7	5.1
	Light	30.5	57.6	43.8	63.1	33.9	62.7	39.0	64.4	30.5	64.4	40.7	57.6
	Negative	-		8.8		5.1		6.8		10.2		25.4	

[1] Reproduced from Kabata and Whitaker, 1981.
[2] W, prevalence of white (young) pseudocysts.
[3] B, prevalence of black (old) pseudocysts.

TABLE IV. Prevalence of *Kudoa thyrsitis* in Georgia Strait hake distributed by body area (in absolute numbers).[1]

	I		II		III		IV		V		VI	
(n = 99)	W[1]	B[2]	W	B	W	B	W	B	W	B	W	B
Heavy	3	7	-	1	-	1	-	1	-	1	-	1
Medium	6	45	4	9	1	21	2	15	-	6	-	4
Light	38	46	25	73	22	73	24	75	7	71	8	70
Negative	-		8		4		6		21		24	

[1] W, prevalence of white (young) pseudocysts.

[2] B, prevalence of black (old) pseudocysts.

TABLE V. Prevalence of *Kudoa thyrsitis* in Georgia Strait hake, distributed by body area (in % of population examined).*

	I		II		III		IV		V		VI	
	W	B	W	B	W	B	W	B	W	B	W	B
Heavy	3.0	7.1	-	1.1	-	1.0	-	1.0	-	1.0	-	1.0
Medium	6.1	45.4	4.4	9.9	1.0	21.2	2.0	15.3	-	6.1	-	4.0
Light	38.4	46.5	27.4	80.2	22.2	73.7	24.5	76.5	7.1	71.7	8.1	70.7
Negative	-		8.8		4.0		6.1		21.2		24.2	

* Reproduced from Kabata and Whitaker, 1981.

TABLE VI. Relationships among infection, protease(s) and texture.

location	infection	n	[1]acid protease	[1]neutral protease	[2]texture
Georgia Strait	K. thyrsitis, $W^{+}B^{\pm}$[3]	54	.86 (.05)	.13 (.01)	15.6 (.49)
"	K. thyrsitis, $W^{-}B^{+}$[4]	44	.35 (.02)	.10 (.01)	20.1 (.54)
West Coast	K. thyrsitis, $W^{+}B^{\pm}$	16	.50 (.04)	.08 (.01)	22.1(1.41)
"	K. thyrsitis, $W^{-}B^{+}$	87	.41 (.02)	.09 (.01)	24.9 (.71)
"	uninfected	30	.41 (.03)	.08 (.01)	23.6(1.06)
"	K. paniformis, $W^{+}B^{\pm}$	95	1.42 (.05)	.80 (.04)	10.2 (.85)
"	K. paniformis, $W^{-}B^{+}$	22	.51 (.05)	.12 (.01)	19.8(1.37)
"	mixed infection	59	.98 (.05)	.36 (.03)	14.9 (.63)

[1]mean values from fish held from 0-5 days on ice or in R.S.W. expressed as O.D. 700 m $\pm$ S.E.

[2]mean values from fish held from 0-5 days on ice or in R.S.W. expressed in kg shear press force $\pm$ S.E.

[3]white pseudocysts present, black streaks present or absent.

[4]white pseudocysts absent, black streaks present.

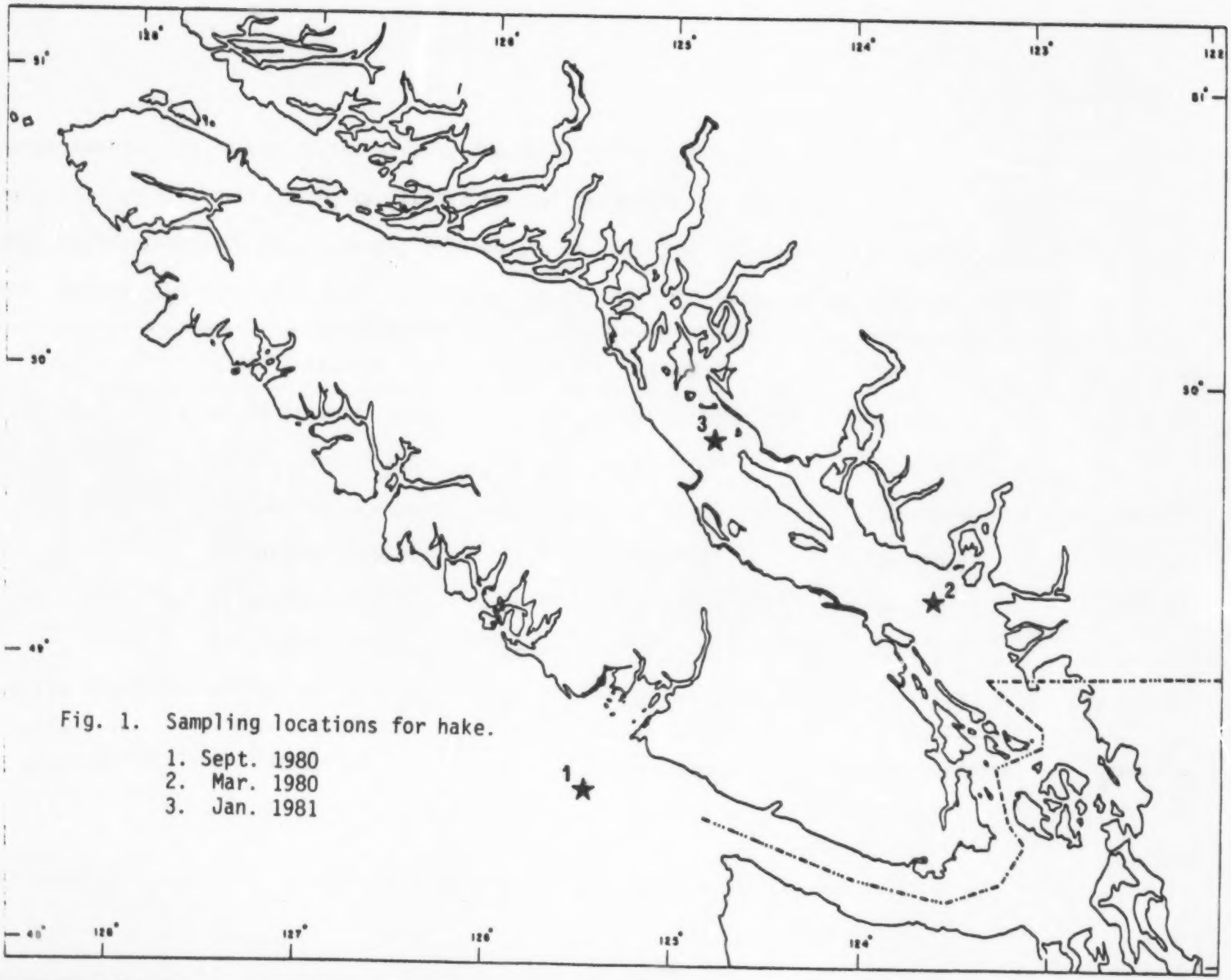


Fig. 1. Sampling locations for hake.

1. Sept. 1980
2. Mar. 1980
3. Jan. 1981

Fig. 2. Proteolysis/temperature at pH 3.8

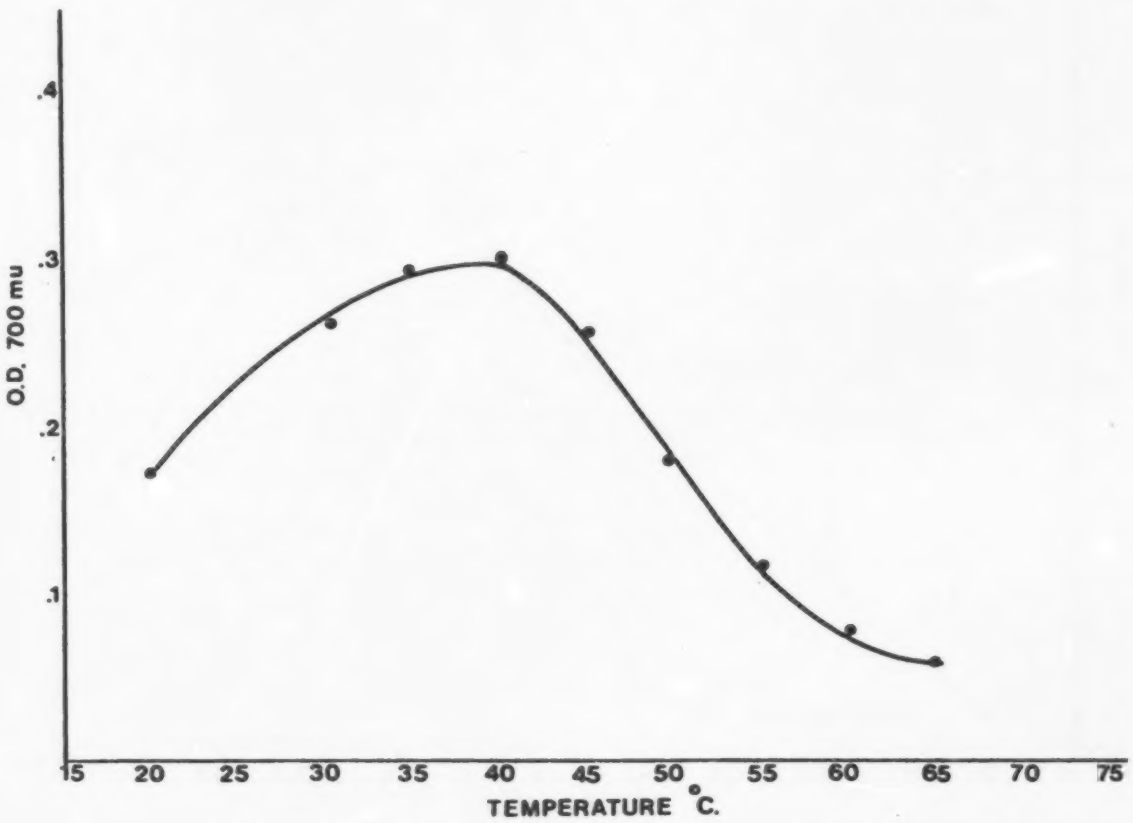


Fig. 3. Proteolysis/temperature at pH 6.7

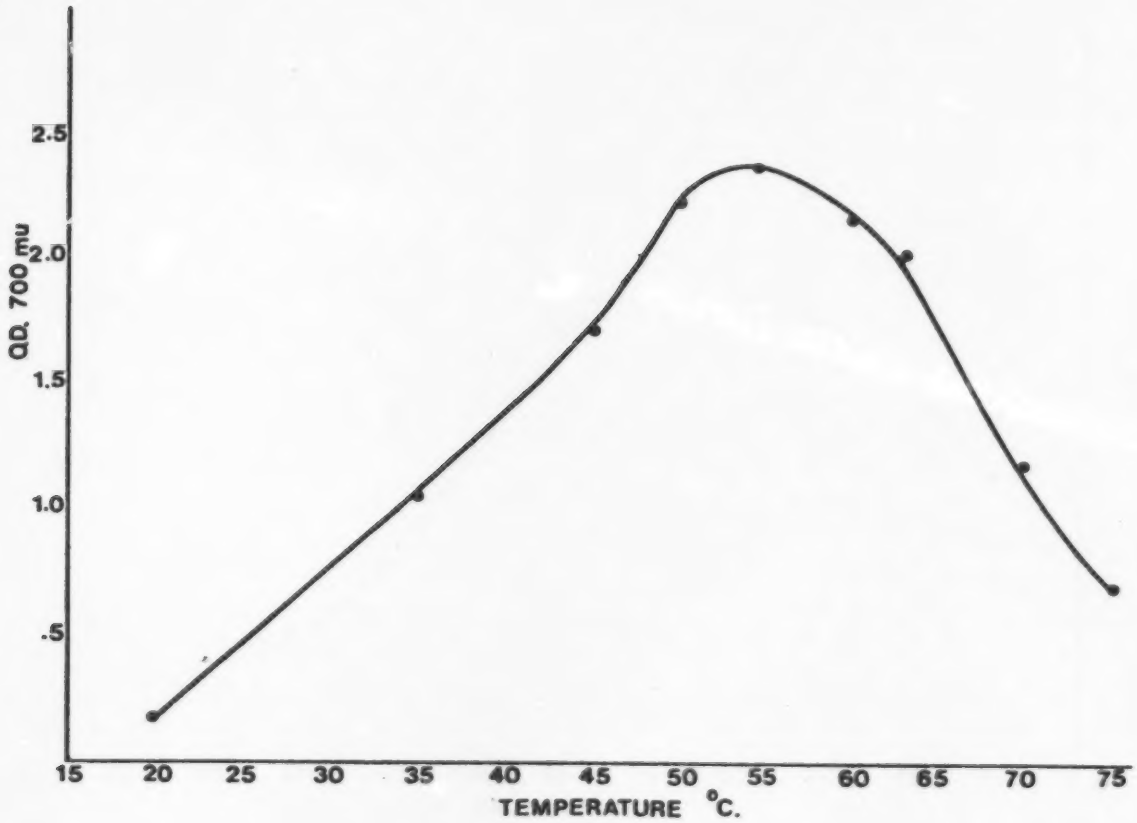


Fig. 4. Proteolysis/pH at 35°C (non-infected)

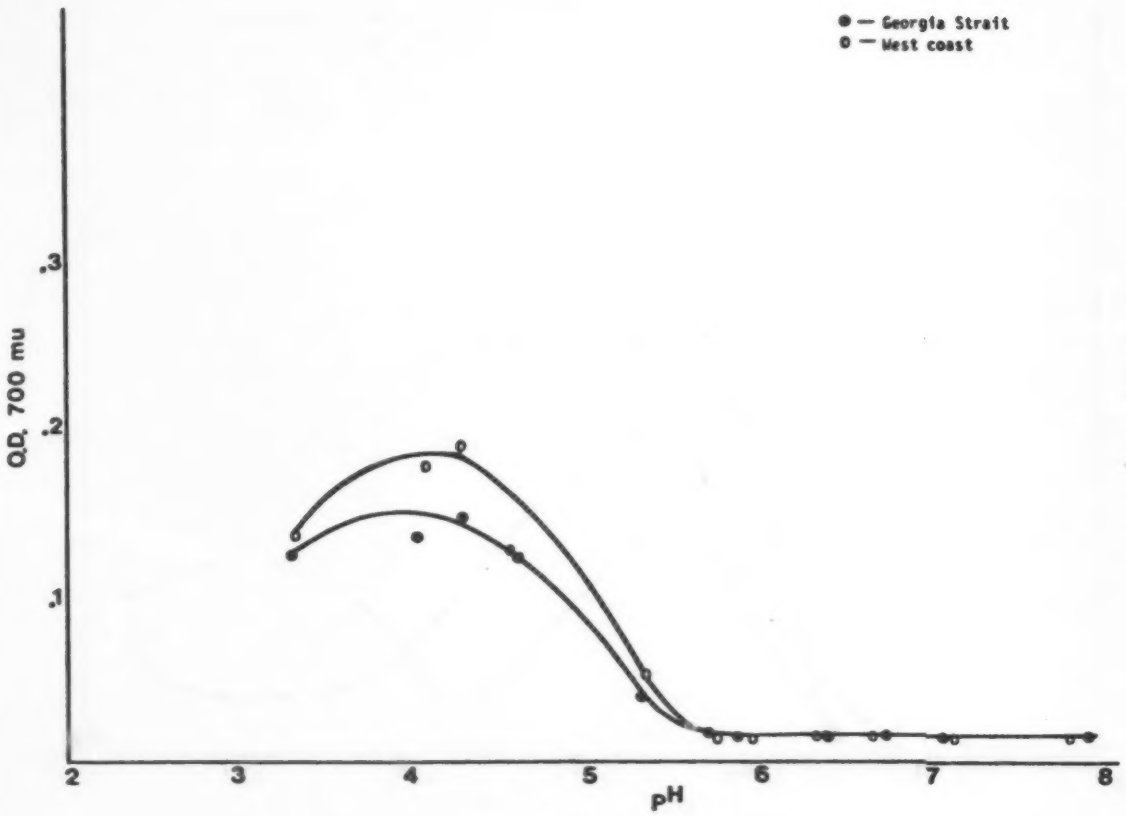


Fig. 5. Proteolysis/pH at 60°C (non-infected)

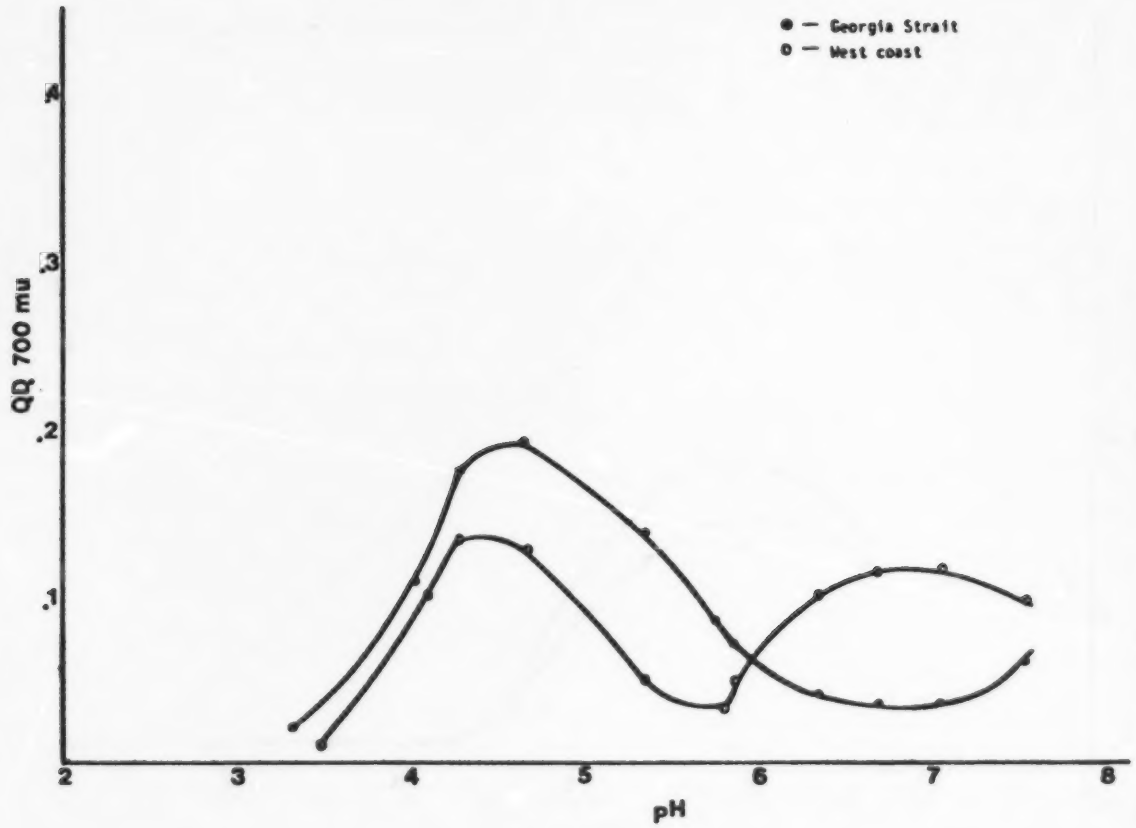


Fig. 6. Proteolysis/pH at 35°C and 60°C Georgia Strait (infected)

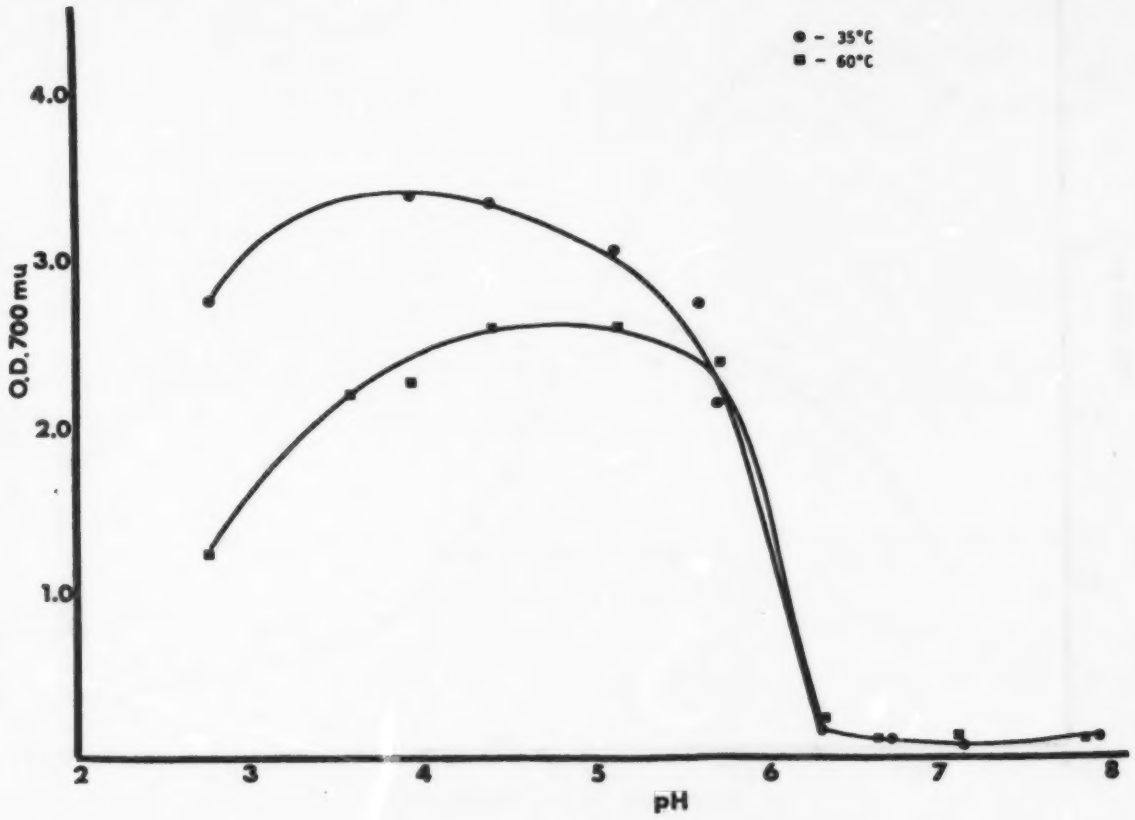


Fig. 7. Proteolysis/pH at 35°C and 60°C West coast (infected)

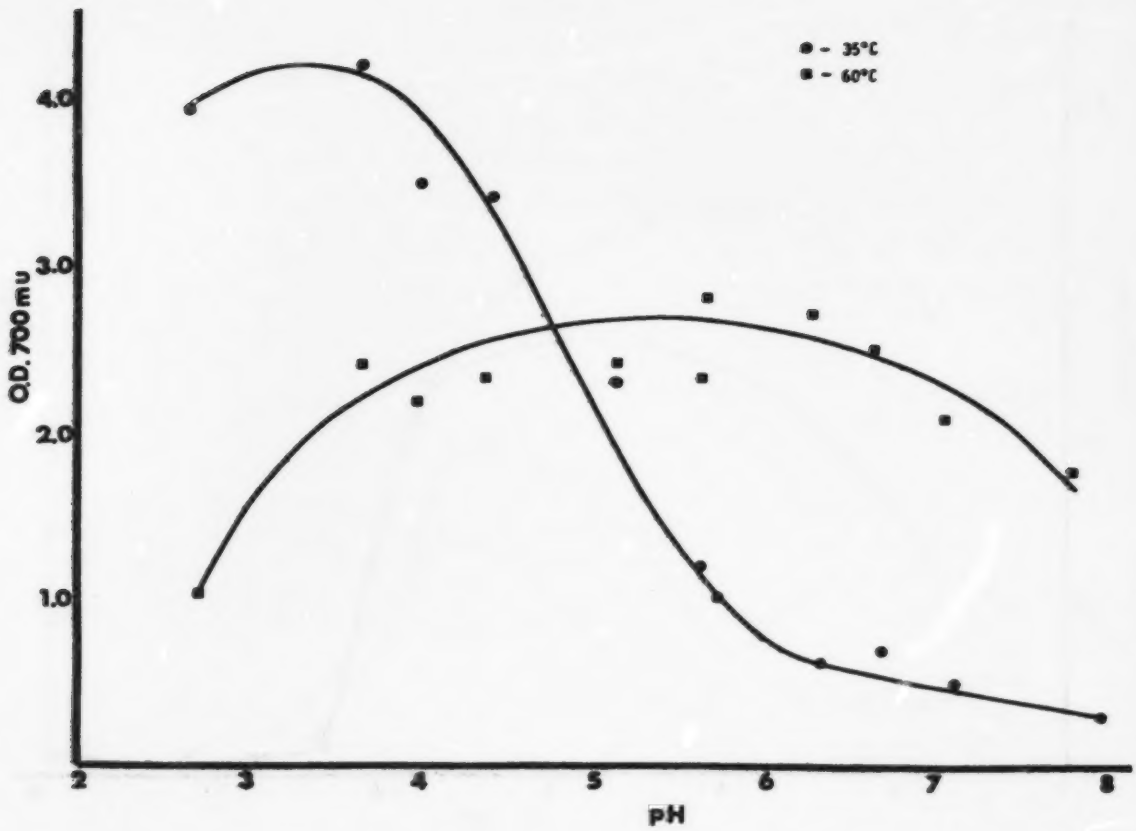


Fig. 8.

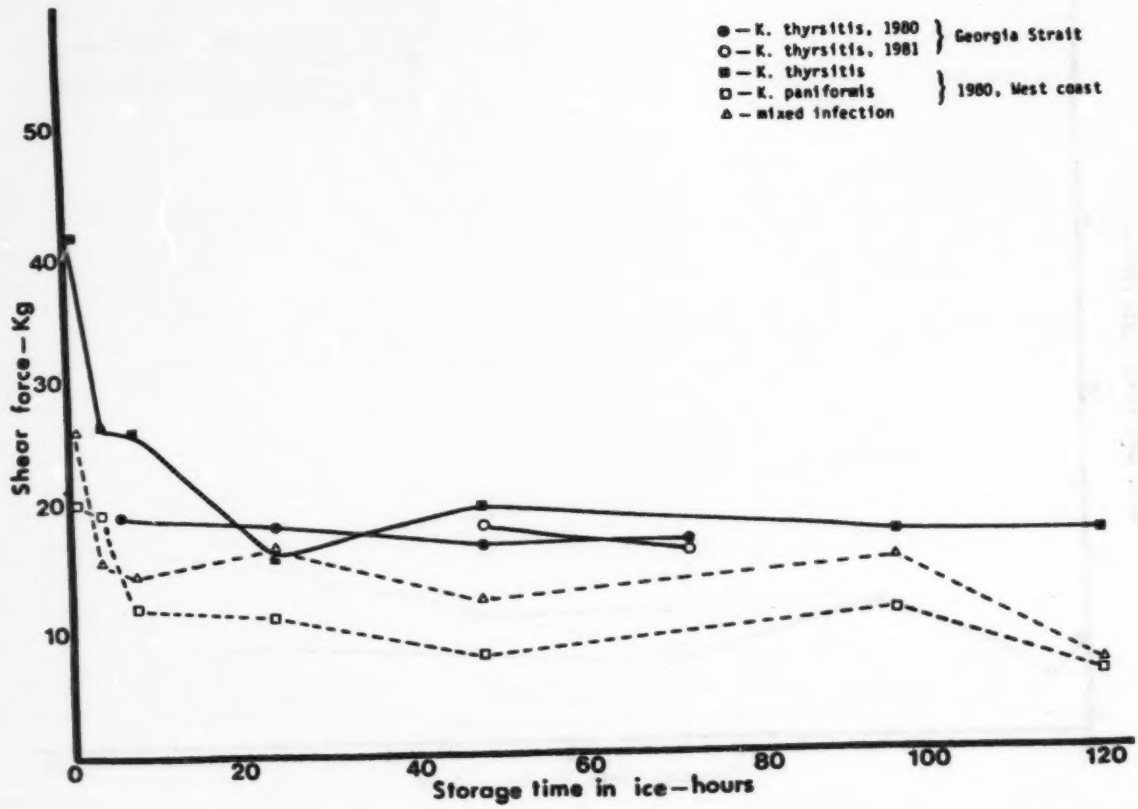

● — K. thyrsitis, 1980
○ — K. thyrsitis, 1981
} Georgia Strait
■ — K. thyrsitis
□ — K. paniformis
△ — mixed infection
} 1980, West coast
Shear force — Kg
50
40
30
20
10
0
20
40
60
80
100
120
Storage time in ice — hours

Fig. 9.

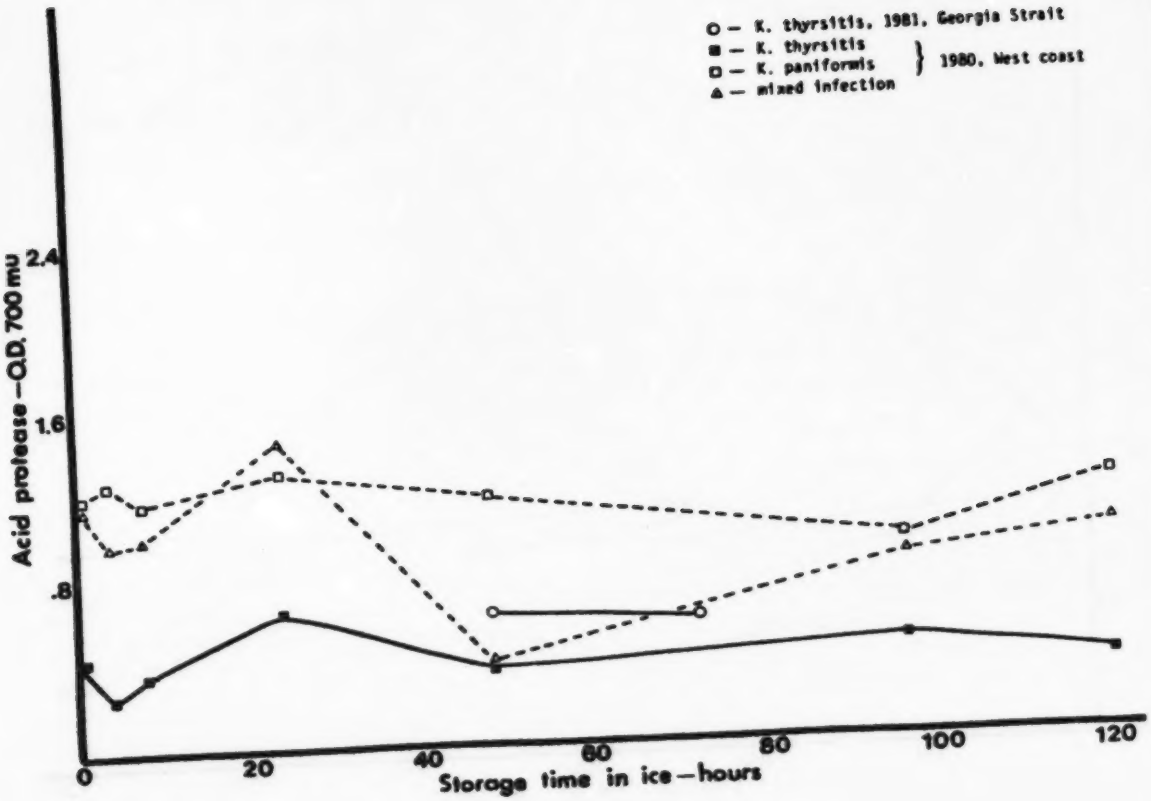

○ — K. thyrsitis, 1981, Georgia Strait
■ — K. thyrsitis
□ — K. paniformis
△ — mixed infection
1980, West coast
Acid protease — O.D. 700 mu
2.4
1.6
.8
0
20
40
60
80
100
120
Storage time in ice — hours

Fig. 10.

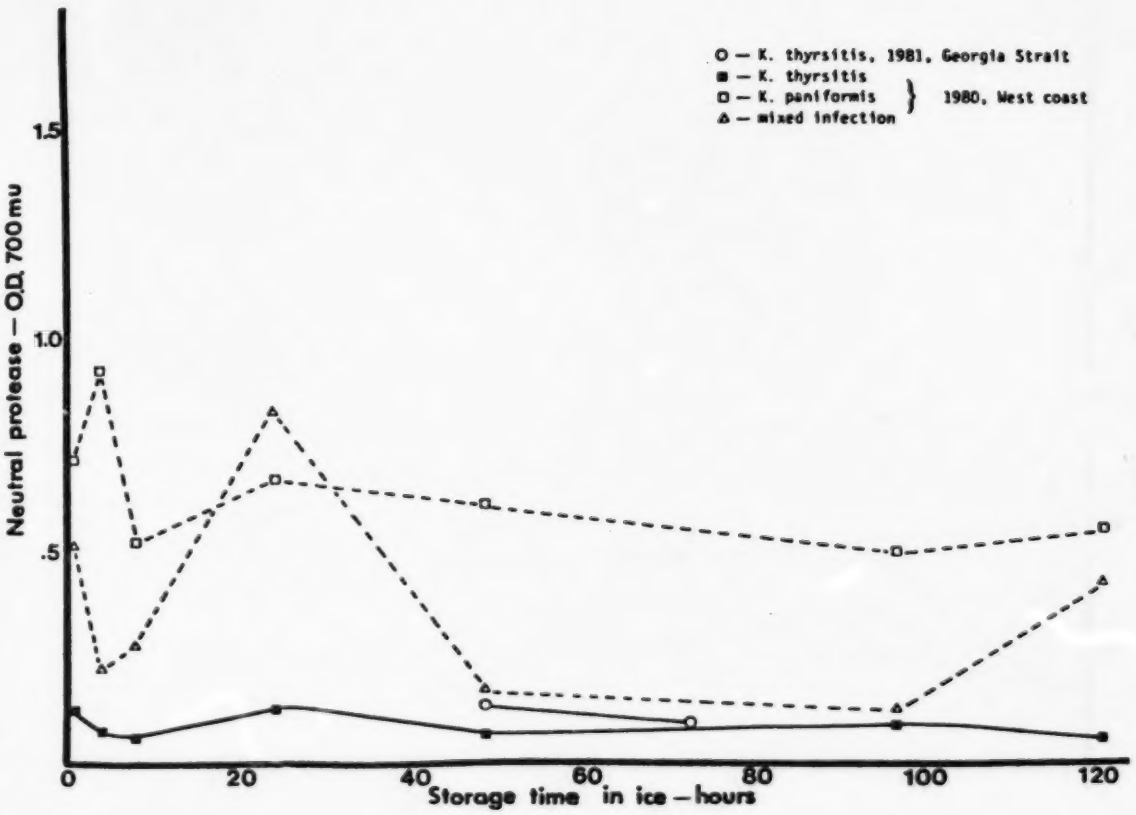

O — K. thyrsitis, 1981, Georgia Strait
■ — K. thyrsitis
□ — K. paniformis } 1980, West coast
△ — mixed infection
Neutral protease — O.D. 700mu
1.5
1.0
.5
0
20
40
60
80
100
120
Storage time in ice — hours

Fig. 11.

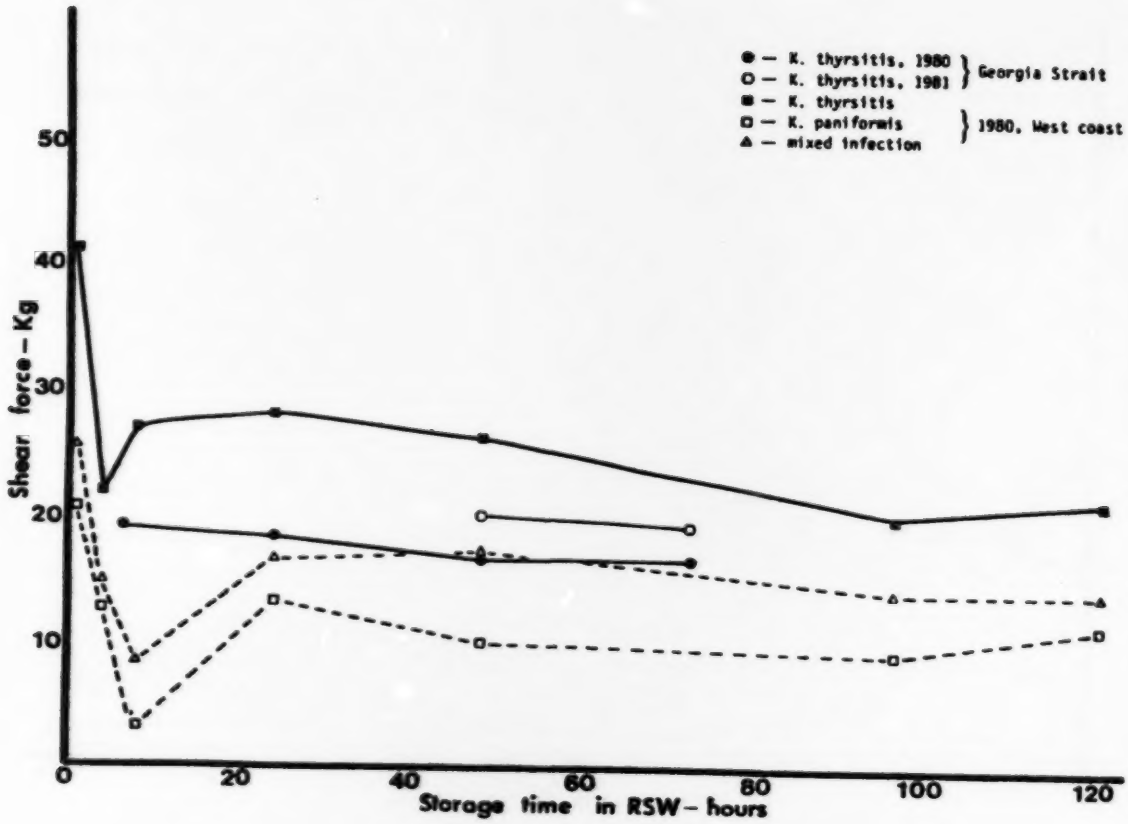

● — K. thyrsitis, 1980
○ — K. thyrsitis, 1981
} Georgia Strait
■ — K. thyrsitis
□ — K. paniformis
△ — mixed infection
} 1980, West coast
Shear force – Kg
50
40
30
20
10
0
20
40
60
80
100
120
Storage time in RSW – hours

Fig. 12.

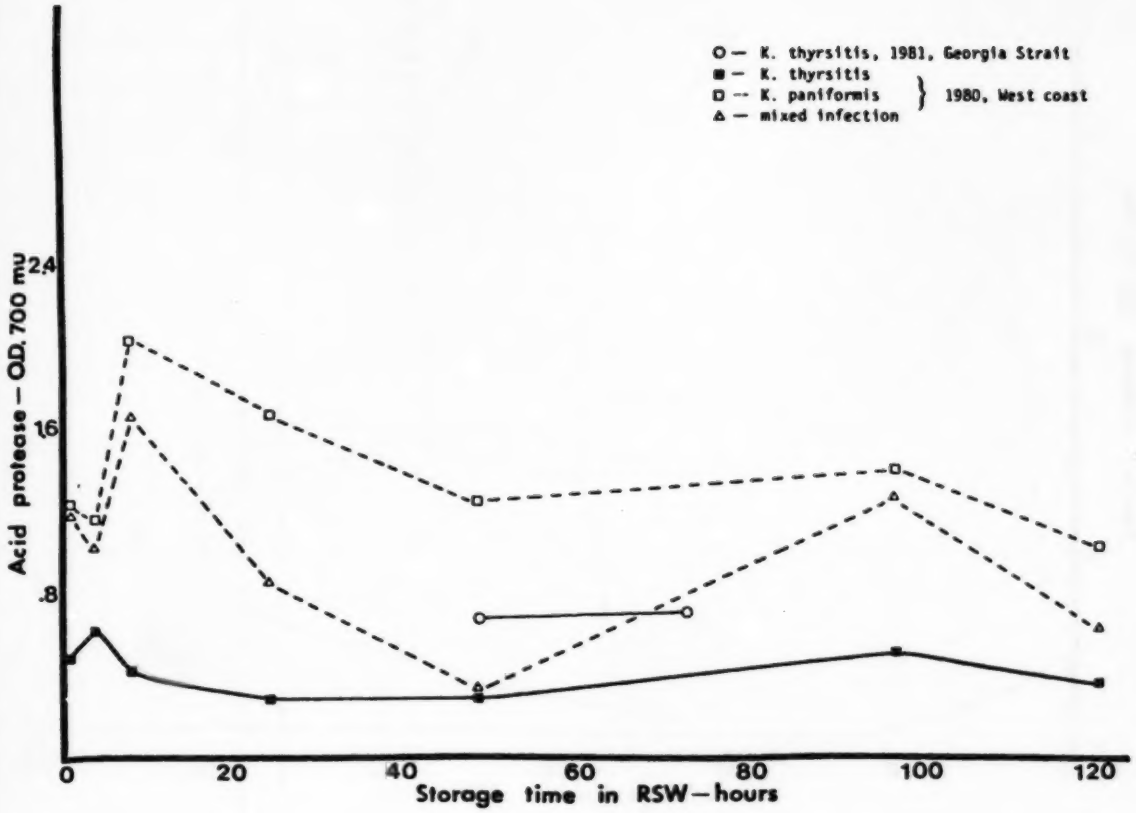

O — K. thyrsitis, 1981, Georgia Strait
■ — K. thyrsitis
□ — K. paniformis } 1980, West coast
△ — mixed infection
Acid protease — OD. 700 mu
2.4
1.6
.8
0
20
40
60
80
100
120
Storage time in RSW — hours

Fig. 13.

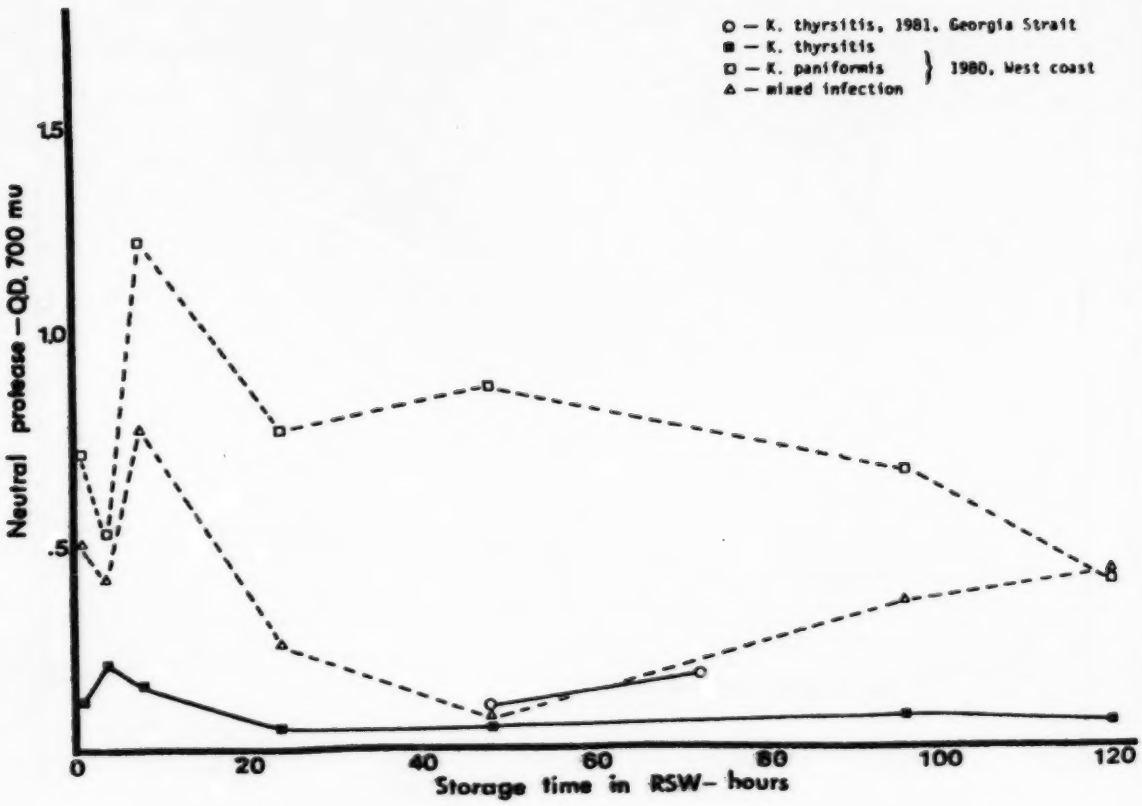
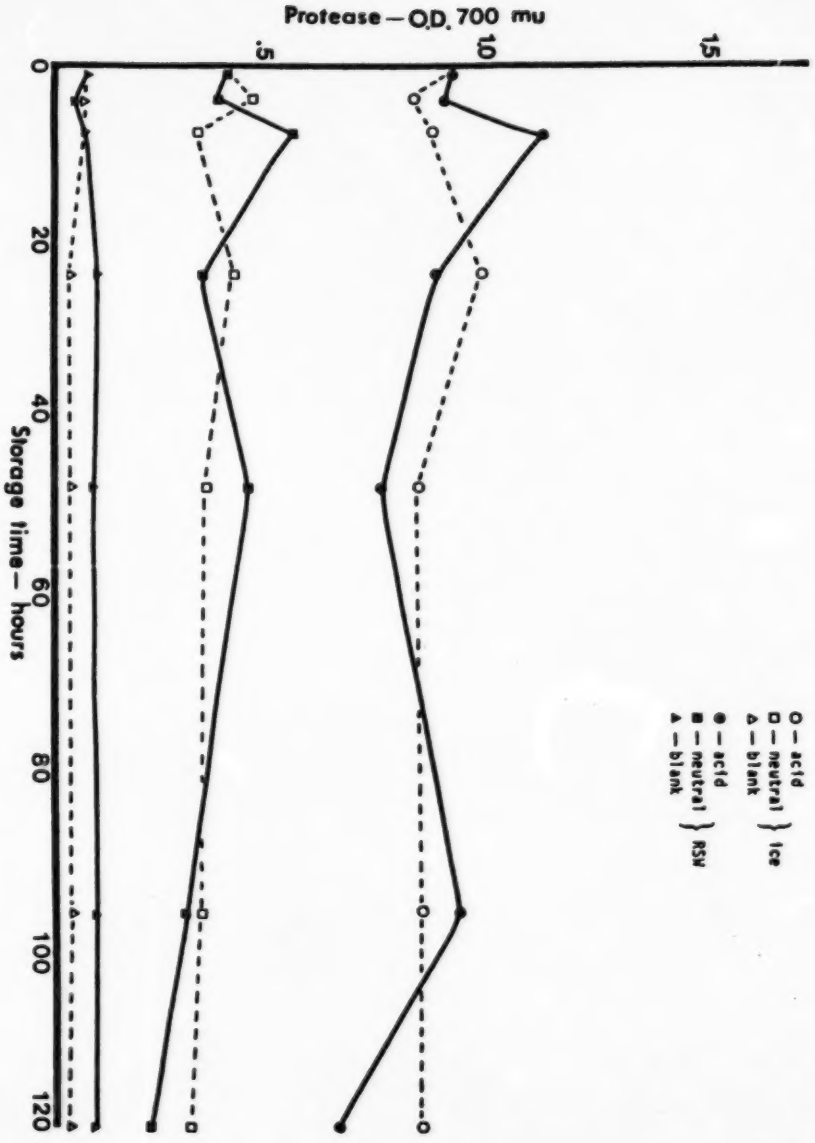

○ — K. thyrsitis, 1981, Georgia Strait
■ — K. thyrsitis
□ — K. paniformis } 1980, West coast
△ — mixed infection
Neutral protease — O.D. 700 mu
1.5
1.0
.5
0
20
40
60
80
100
120
Storage time in RSW — hours

Fig. 14.


Protease — O.D. 700 mu
.5
1.0
1.5
0
20
40
60
80
100
120
Storage time — hours
○ — acid
□ — neutral } ice
△ — blank
● — acid
■ — neutral } RSW
▲ — blank